

CONTRIBUTIONS TO OUR KNOWLEDGE OF SOIL-FERTILITY.

iv. The Agricere and Bacteriotoxins of Soil.

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In the first paper of this series, I showed that, in opposition to Russell and Hutchinson, there were in soils certain toxins which restrained the growth of the soil-bacteria. These were partly or entirely destroyed by heat, by sunlight, and by storage, especially in aqueous solution. Furthermore, I showed that soil contained a fatty or waxy substance, *agricere*, which was dissolved and carried to the surface of the soil by certain fat-solvents. These solvents are also disinfectants, and this property of destroying vegetating bacteria is probably responsible for obscuring their other property of dissolving fatty bodies.

The research originated in the disbelief of one of the steps which led Russell and Hutchinson to think of protozoa. It was against all bacteriological experience that bacterial toxins should be absent from soil; and it was only by assuming or claiming that no toxins were in soil, that these investigators were led to believe that phagocytic protozoa were responsible for the limitation of the bacterial content of soils.

That the soil-protozoa should play a part in checking the multiplication of bacteria in the soil is very feasible, and many of the experiments recorded by Russell and Hutchinson point to their activity. But it does not follow that the protozoa are alone responsible for the limitation in the numbers of bacteria in soil, as Hall* would lead us to believe. Indeed, Russell and Hutchinson are careful to say that they do not wish to imply that the removal of the large organisms is the only cause of the improvement in soils effected by partial

* Hall, Chem. Soc. Ann. Rep. 1909, 187.

sterilisation. The possibility of other factors was mentioned, and an instance given in a nitrogenous substance very soluble in toluene, the distribution of which would be affected by the toluening. This toluene-soluble substance is so suggestive of *agricere*, that it is probable that further investigation would show that the claim to its being nitrogenous, has been founded upon insufficient data.

Agricere is presumably derived from the substances "soluble in ether" of plant-roots, stubble, and similar organic matter, which have slowly decayed, and have become incorporated with the soil. The rate of decay of the cellulose and other carbohydrates of the vegetable matter will naturally be more rapid than that of the fatty substance, so that, ultimately, the latter will become concentrated upon the remains of the former, and, by saturating the residual fermentable organic matter, the *agricere* will act as a preservative. I have referred to this condition as a "waterproofing." By the removal of *agricere*, the soluble portions of the organic matter will more readily diffuse out, and the soil-bacteria will more readily get into contact with the fermentable fragments of organic matter. From its presumed origin, the *agricere* should be associated with the organic matter, and should, to a certain extent, be proportional to it. The amount in soils is small, but the quantity of organic matter is, as a rule, also small. The effect of treatment with antiseptics is much as if the crop had received a nitrogenous manuring, which indicates an accelerated decomposition of the organic matter, such as would occur if the *agricere* were segregated.

I believe that to be the behaviour of the *agricere* in the soil. With regard to its presence, there can be no doubt, for Schreiner and Shorey* have, simultaneously with me, shown the presence of the glycerides of fatty acids and of paraffin-hydrocarbons in soils. These are the *agricere*-components which I have referred to as saponifiable and non-saponifiable.

* Schreiner and Shorey, *Journal American Chemical Society*, Jan. 1911.

These authors agree with me in considering that the *agricere* is derived from the remains of the organic matter of plants.

With the removal or segregation of the *agricere*, the soluble matter will more readily diffuse out from the soil-particles, and as this may be divided into toxic and nutritive substances, the benefit derived from the removal will depend, in the first place, upon the relative preponderance of the one or the other of these. In experiments with soil-extracts, the toxin* generally predominates while, in soils, the nutrients preponderate.

It is difficult to explain the difference between the behaviour of the moist soil and of the extract. A plausible explanation may be found in the relative quantities of water used in the two methods, and in the faint acidity† of the soil. In the soil-tests, the water varied from one-tenth to one-quarter of the weight of the soil, just enough to make it damp; while, in the extracts, an equal weight of water was employed. With the smaller proportion of water, the acidity of the soil would not be so much weakened, and, taken in conjunction with the longer exposure, more of the presumably less soluble nutrients would be dissolved. This reasoning, however, does not appear to hold.

Twenty gr. portions of garden-soil were placed in deep Petri dishes, and to each, 2 cc. of a suspension of *Bac. prodigi-*

* I have used the words "toxin" and "toxins" indiscriminately. Doubtless there are as many varieties of toxin as there are groups of bacteria in the soil. It is known that the toxins of one group may not be toxic to another group, but, so far as these experiments go, *Bac. prodigiosus* behaves to the soil-toxins and nutrients like the ordinary soil-bacteria, and, since its growth is rapid and the colonies easily detected, it acts after the manner of an indicator.

† From the literature upon the subject, one is led to believe that all normal soils are faintly alkaline. Mr. R. S. Symmonds, of the Department of Agriculture, drew my attention to the fact that the majority of soils are acid to litmus, and this holds for the soils I have tested in New South Wales. The method of testing consists in stirring the soil with a small quantity of water, thus forming a paste; and, after from 5 to 10 minutes, pressing a piece of litmus paper upon the mass.

giosus were added, together with varying amounts of water. In 20 hours the following increases were obtained.

EXPERIMENT i.

20 gr. of soil with 2 c.c. of suspension and addition of		10 bacteria became
No water.....	= 10 % added.....	29
3 c.c. of water..	= 25 % added	40
8 c.c. of water....	= 50 % added	55
18 c.c. of water....	= 100 % added.....	75

The soil-bacteria were not influenced by the water, as approximately the same number of white colonies developed in all tests in 20 hours.

An attempt to solve the matter was made in another way. An extract of soil was prepared by using 200 c.c. for 500 gr. The extract, however, was toxic; 1,000 bacteria became reduced overnight to 35, while, in a water-control, they increased to 4,050.

Dilution of the soil-extract alters the relation between the toxins and the nutrients, and, in place of having a toxic action, the diluted solution becomes more or less nutritious. This is seen in Experiments iv., and xxiii,

In considering the action of the volatile disinfectants, it is necessary to know if they have any action upon the soil-bacteriotoxins, either destroying or dissolving, and subsequently translating them to other parts of the soil, as in the case of *agricere*.

The Action of Fat-solvents upon the Soil Bacteriotoxin.—In order to determine if the bacteriotoxin were soluble in fat-solvents, ten experiments were made with chloroform and with ether. The general outcome was that neither of these disinfectants had any solvent action. When the chloroform-extract of soil was evaporated in a current of air, and the waxy residue taken up with warm water, a slight toxic action was noted; but if the residue was treated with warm saline, the solution was found to be slightly more nutritive than a saline control. Traces of chloroform have no pronounced bacteri-

cidal action, as will be seen on p.686. Ether behaved like chloroform in dissolving no toxin, and since these two disinfectants have no solvent action, we are probably justified in concluding that other fat-solvents are similarly inactive.

The solvents, furthermore, have apparently no antitoxic or destructive action upon the toxin, if we judge by the behaviour of the soil-extracts after treatment of the soil with chloroform and with ether. Two soils were tested, and the results are shown in the following tables:—

EXPERIMENT ii.

Garden-soil 200 gr. : 200 c.c. : 1 hr.	1,000 bacteria became
Aqueous extract after treatment with chloroform	0
The same extract heated.....	3,850
Saline extract of the soil-residue from above.....	23
The same heated.....	6,300,000

EXPERIMENT iii.

Hawkesbury soil No.4. 100 gr. : 200 c.c. : 1 hr.	1,000 bacteria became
Aqueous extract after treatment with chloroform	0
Aqueous extract after treatment with ether.	0
Aqueous extract without previous treatment	200
Aqueous control (no soil).....	7,600

EXPERIMENT iv.

Aqueous extract of soil. Soil No.4.	1,000 bacteria became					
	<i>Bac. prodigiosus.</i>				Soil-bacteria.	
	Ratio = 100 gr. : 200 c.c. : 1 hr.				200gr.:200c.c.:1hr	
	diluted 1/2.		diluted 1/4.		undiluted.	
	unboiled	boiled	unboiled	boiled	unboiled	boiled
After treatment with chloroform	0	642,000	1,600	207,000	930	20,270
After treatment with ether	0	174,000	700	532,000
Untreated.....	0	563,000	2,180	495,000	1,200	15,510
Aqueous control...	5,100		5,600		1,100	

From these experiments, it is evident that the soil-bacterio-toxin is not destroyed, and is probably unaffected by chloroform or ether. As it is not dissolved by these solvents, it follows that the numerical increase, which follows the treatment of the soil with these disinfectants, is not caused by any alteration in the bacteriotoxin already present in the soil.

The Effect of Reaction of the Medium upon the Toxic Action of Soil-Extracts.—Two sets of experiments, with gradually increasing amounts of lactic acid and of sodium carbonate, showed that a neutral or faintly acid solution favoured the growth of *Bac. prodigiosus* in filtered soil-extracts, but that the reaction had no influence upon the toxin, was shown by the control-experiments in which no toxin was contained, behaving in a precisely similar manner.

The Soil-Bacteriotoxin is not Volatile.—Beyond the fact that the soil-bacteriotoxin is toxic to bacteria, is thermolabile, is destroyed by sunlight, and is insoluble in chloroform and ether, we know little about it. It appears to be non-volatile, for when air-dried soil was heated at 75° C. in a slow current of air under diminished pressure, the few drops of condensed water that were collected possessed no toxic properties.

Action of Rain.—In the soil, the bacterial growth is weakened by the toxin and strengthened by the soil-nutrients, and the fertility, so far as these factors are concerned, will depend upon the equilibrium established between the two. One of the effects of rain is to dissolve the toxin, and carry it into the subsoil, where it may decay after the manner of the toxin in filtered extracts. The Hawkesbury soil, No. 4, was undoubtedly less toxic after heavy rains in January, 1911, than it was in October, 1910, and the earlier part of the same year.

This observation led to an experiment being tried to test the distribution of the toxin after moistening the soil with water, similar to what would occur with a heavy shower of rain. A kilogram of soil was placed in a wide glass jar (the

bottom of a moist chamber), and, in this, the soil showed a depth of two inches. The soil was then thoroughly wetted by sprinkling from above with distilled water, and allowed to dry in the air. The top half was separated from the lower, and both portions were exposed to the air for a day. Suspensions of *Bac. prodigiosus* were added to the soil and to its extract, as in previous experiments.

EXPERIMENT V.

—	1,000 bacteria became		
	Hawkesbury No. 4.		Garden-soil.
	Soil.	Extract.	
Top soil.....	3,600	520	2,900
Bottom soil	3,000	123	2,000

The greater toxic power of the bottom soil appears to show that the toxin is carried down by the water percolating from above.

Treatment of the Soil with Non-Volatile Disinfectants.—If the action of the volatile disinfectants is solely to kill phagocytic protozoa, the non-volatile disinfectants should behave in a precisely similar manner, while the fatty bodies in the soil should be unaffected. To test if this reasoning was good, portions of a soil were treated with solutions of 5 % phenol, 0.1 % mercuric chloride, 1 % potassium bichromate, and 1 % copper sulphate, for a day. They were then washed and dried. A portion of each was treated with chloroform, and compared with the untreated portion. Unfortunately all the portions were toxic, and the whole of the added bacteria were killed off. Evidently the non-volatile disinfectant adheres tenaciously to the soil-particles, and resists removal by a moderate quantity of water. Upon again moistening the soil, the residual disinfectant is dissolved, and checks bac-

terial growth. Potassium permanganate was not toxic, but the soil-particles had apparently become covered with the reduced salt, as it became very absorbent; there was no difference between the treated and the untreated portions.

The Action of Chloroform-Water upon the Growth of Bacteria in Soil.—As a rule, substances that are toxic in comparatively large doses, are generally stimulative in comparatively small quantities, and it is just possible that traces of the volatile disinfectant adhere to the soil-particles, and stimulate the growth of bacteria. It had been shown, in a preliminary experiment, that small quantities of chloroform did not prohibit the growth of bacteria, and an experiment was made to see if there could be any stimulation. Several 20 gr. portions of soil were put into bottles, and mixtures of chloroform-water and sterile tap-water in varying proportions were added, along with a suspension of *Bac. prodigiosus*. The total quantity of fluid added was 12 c.c. to each bottle. The tests were incubated overnight at 30°, and, on the following day, dilutions were made, and the number of bacteria determined.

EXPERIMENT vi.

Soil with chloroform-water.	10 cells of <i>Bac. prodigiosus</i> became	Rough relative analyses of the other colonies upon the plates.			
		Fluorescent colonies.	Flat, dry colonies.	Moist, raised colonies.	Small colonies.
66%	4	1	22	1	8
50%	8	2	15	3	15
33%	29	9	16	2	43
16.7%	42	0	12	1	100
None	84	0	15	1	38*
„	none added	3	12	2	148

So far as *Bac. prodigiosus* is concerned, it is seen that chloroform-water restrains the growth, but that a multiplication occurs in the presence of one part of chloroform-water

* The increased growth of *Bac. prodigiosus* had checked these bacteria.

and two parts of tap-water (33 %). There is, however, no evidence of any stimulating influence of the volatile disinfectant.

The Search for an Antitoxin.—The knowledge of the existence of a toxin in the soil naturally leads to the desire to find an antitoxin or substance which, when applied after the manner of a fertiliser, will favour the growth of bacteria by neutralising the toxin. Practically this must be cheap, and easy of application. So far as economy is concerned, we have seen that exposure to the sun's rays diminishes the toxicity, and doubtless the benefits that accrue from working the soil are, in part, traceable to this fact. It is possible that this, together with the natural decay, is the only economic method of combating the accumulation of the toxic products of bacteria.

But an enhanced fertility has been obtained, in certain cases, by the use of substances which are not generally considered as fertilisers, inasmuch as they do not contain the customary nitrogen, phosphoric acid, potash or other constituent taken up by the plant in quantity. For example, ferrous sulphate and manganese sulphate have been used as manures, in some cases with advantage to the crop; copper salts also, when used as fungicides, have generally a distinct action in increasing the growth of plants. It is possible that these may act indirectly as toxin-destroyers.

To ascertain the action of saline substances as antitoxins, two methods might be employed in the laboratory. They might be added to the soil itself, or to an extract. In the latter case, the antitoxic action would probably be more pronounced, for the reason that the toxic action is more prominent. Both methods were tried, and the substances that were tested were copper sulphate, copper sulphate followed by lime-water, manganese sulphate, ferrous sulphate, ferric chloride, sodium phosphate, ferrous sulphate and lime-water with air blown through until all the iron had been oxidised, aluminium sulphate, superphosphate, sodium sulphite, sul-

phuretted hydrogen, and sodium thiosulphate. Some of these, as copper sulphate, were themselves toxic, while the others, as superphosphate, simply acted as stimulants after the manner of magnesium and potassium sulphates, as already noted. The only salt that gave any promise of possessing any degree of antitoxic power, was sodium thiosulphate, and it was further investigated. The following four experiments are given as showing the general result in solutions of the extract, and in the soils themselves.

EXPERIMENT vii.

	1,000 bacteria became	
	No thiosulphate.	Thiosulphate, 0·017%.
Dilute extract of stored garden-soil.....	23,000	108,000
Dilute extract of Hawkesbury soil No.4.....	1,000	1,143,000
Water-control.....	1,400	10,000

EXPERIMENT viii.

	1,000 bacteria became
Extract of soil No.4.....	56
Extract of soil No.4, boiled.....	3,700
Extract of soil No.4 with thiosulphate, 0·17%.	5,600,000
Water-control.....	14,000

EXPERIMENT ix.

20 gr. soil + 2 c.c. suspension + 2 c.c. 0·5% thiosulphate or water.	1,000 bacteria became	
	Water.	Thiosulphate.
Hawkesbury soil No.4.....	40	105
" " " ".....	60	109
Stored orchard-soil.....	10	48
Stored garden-soil.....	21	32
Sand.....	13,900	19,200

EXPERIMENT X.

20 gr. soil + 1 c.c. suspension + 1 c.c. thiosulphate or water.	Thiosulphate, %, in water added.	1,000 bacteria became
Hawkesbury soil No.4	0	3,100
" " "	0	2,800
" " "	0.01	2,950
" " "	0.05	4,750
" " "	0.1	5,500
" " "	0.2	5,450
Sand	0	130,000
" "	0.2	540,000

Although the results obtained with the extracts (Experiments vii. and viii.), especially of the Hawkesbury soil, raised the hope that some neutralisation of the toxin had occurred, yet the findings with the soils themselves (Experiments ix. and x.) indicate that the bacterial increase was, in all probability, caused by the stimulation of the salt. The toxin is probably unaffected.

The Action of Heat and of Fat-Solvent upon Soils.—In endeavouring to demonstrate the action of fat-solvents in removing the agricere from soils, it is necessary to eliminate any disturbance produced by the bacteriotoxins and by protozoa.* This would appear to be a simple matter, since both of these are destroyed by heat. Unfortunately, as the natural toxins are destroyed, the heat-toxins of Pickering are developed, and a soil which has been heated for some time, becomes exceedingly toxic. This will appear evident from the consideration of the following experiments. In the tables, I have calculated the increase from 10 original cells of *Bac. prodigiosus*, the micro-organism used in the experiments,

* When a soil has been air-dried and stored in that condition for any length of time, it is exceedingly probable that the phagocytic protozoa will have been destroyed, or so weakened that they will be unable to become sufficiently active to exercise their phagocytic functions within the time, viz., 20 hours, occupied in the experiments. But on account of the uncertainty of their being really inactive, means had to be adopted to ensure their inertness.

which were made in the manner already described* ; 20 grms. of soil being moistened with 2 c.c. of suspension. The soils were heated at 100°-105° C.

EXPERIMENT xi.

Hawkesbury soil No.4 stored 2 months.	10 bacteria became	
	Untreated.	Treated with chloroform.
Not heated	51	307
Heated 1 hour.....	115	108
Heated 2 hours.....	97	12
Heated 4 hours.....	3	8
Heated 6 hours.....	0·3	0

EXPERIMENT xii.

Garden-soil.	10 bacteria became	
	Untreated.	Treated with chloroform.
Not heated	75	1,850
Heated 1 hour.....	10,900	4,170
Heated 2 hours.	18,700	6,250
Heated 4 hours.....	3,020	[75]

EXPERIMENT xiii.

Good arable soil stored 9 days.	10 bacteria became	
	Untreated.	Treated with chloroform.
Not heated	15	785
Heated 1 hour.....	43	30
Heated 2 hours.	16	1
Heated 4 hours.....	0	0

* These Proceedings, 1910, p.813.

These experiments indicate (and the experimental error being considerable, one cannot consider them other than as an indication) that the action of heat and of chloroform is complex. There is a destruction of a natural toxin and the production of a heat-toxin in both the untreated and treated sets. In the chloroformed sets, the action of the natural toxin is masked by the greater diffusion of the nutrients and of the heat-toxins. The latter apparently diffuse more easily out of the particles of treated than out of the untreated soils.

Taken as a whole, the experiments show that the fat-solvent has a pronounced action in liberating the nutrients of unheated soil, and in liberating the toxins of heated soils. The continued action of heat is to destroy the natural toxin, and to produce more and more heat-toxin.

The action of carbon bisulphide is different from that of chloroform, inasmuch as the heat-toxin is either not so diffusible, or it is largely destroyed. This will be seen from the following. As we have no reason for believing that the heat-toxin can be less diffusible, we may conclude that it is more or less destroyed. There is a doubt, however, about the purity of the carbon bisulphide; some samples that I obtained were toxic, and it may be that the solvent used in these experiments contained a nutrient.

EXPERIMENT XIV.

Garden-soil.	10 bacteria became			
	<i>a</i>		<i>b</i>	
	Untreated.	Treated with carbon bi-sulphide.	Untreated.	Treated with carbon bi-sulphide.
Not heated	127	780	22	350
Heated 1 hour.....	1,377	3,240	2,720	3,100
Heated 2 hours.....	3,677	6,340	9,000	18,520
Heated 4 hours.....	3,380	7,850	6,630	[160]

EXPERIMENT xv.

Hawkesbury soil No.4 stored 2 months.	10 bacteria became	
	Untreated.	Treated with carbon bisulphide
Not heated.	71	550
Heated 1 hour at 105°.	140	440
Heated 2 hours at 105°.	5	130
Heated 4 hours at 105°.	7	21

In the experiments which have been recorded, the development of the heat-toxins and their solubility in water prevented the action of the agricere being clearly shown. Accordingly, another method of endeavouring to demonstrate the solvent action of the disinfectants upon the agricere was adopted. This had for its principle the pasteurisation of the soil. It is exceedingly unlikely* that the protozoa can survive exposure to a moist heat at 75° C., for 10 minutes, and, in view of the previous experiments, that any appreciable amount of heat-toxin will be developed.

A garden-soil was moistened with water, and heated in the water-oven. In two hours, the temperature of the soil, as recorded by a thermometer with its bulb in the middle of the soil, reached 75°, and 10 minutes later it had risen to 78°. The dried layer of soil on the top was rejected, and the lower moist soil was spread out upon a sheet of glass, and allowed to cool and dry in the air. Portions weighing 20 gr. were taken, and tested with and without previous treatment with ether (Merck).

* Russell and Golding, Journ. Soc. Chem. Ind. xxx. 1911, p.741, say that the protozoa are completely destroyed at 60°.

EXPERIMENT xvi.

	10 bacteria became
Pasteurised soil	255
Pasteurised soil treated with ether	1,190

As there could be no phagocytes present in the pasteurised soil, and since it has been shown (pp. 682-684) that ether has no action upon the soil-toxin, the increased growth from the ether-treatment can be ascribed only to the increased solubility of the nutrients, resulting from the segregation of the *agricere*.

The action of the fat-solvent was again tested upon two soils, which had been obtained from the Hawkesbury Agricultural College. They were designated "good" and "medium." After being moistened with water, they were heated in an air-oven, as in the previous experiment. The temperatures in the middle of the soils reached 75° in an hour, and was 79° ten minutes later. The top layers were rejected, and the soils were spread out to cool and dry.

EXPERIMENT xvii.

	10 bacteria became	Soil-bacteria in 0.0001 gr. of test soil after 3 days at 28°
Good soil untreated	32	—
Good soil pasteurised	580	59
Good soil pasteurised and treated with chloroform	8,015	102
Medium soil untreated	52	—
Medium soil pasteurised	185	20
Medium soil pasteurised and treated with chloroform	31,750	53

With regard to the soil-bacteria, 20 gr. portions of the soils were, after treatment, moistened with sterile water, and

incubated at 28° for three or four days. The numbers represent the colonies that grew upon plates seeded with the suspension from 0.0001 gr. of soil. With a short incubation period, one cannot expect to get true comparative results when there is a difference in the treatment of the soil-tests. The above result is probably exceptional, and chanced to agree with the tests made with *Bac. prodigiosus*. A soil which has been pasteurised and treated with chloroform should contain fewer bacteria than a soil which has only been pasteurised; the double treatment should further reduce the number of bacteria. Thus, at the time when the soil is moistened, there should be a greater number of micro-organisms in the pasteurised than in the pasteurised and chloroformed soil. This will give the former a favourable start, and a considerable time, weeks perhaps instead of days, will elapse before the effect of the soil-treatment can really be seen. For this reason, the experiments with *Bac. prodigiosus* are to be preferred, as indicating the true response of the soils to the various methods of treatment.

After noting that Russell and Golding had said that the phagocytic protozoa are completely destroyed at 60°, the air-dried soils were heated at 65°-68° for 10 minutes, and tested as in the previous experiment.

EXPERIMENT xviii.

	10 bacteria became	
	Garden-soil.	" Good " soil.
Untreated.....	242	248
Heated at 65°	1,700	520
Heated at 65° and chloroformed...	9,880	2,440

Further sets of experiments were made with the idea of testing the various solvents, and the results confirmed those previously obtained.

EXPERIMENT xix.

Pasteurised garden-soil.	Bacteria in 0·0001 gr. of soil.		<i>B. prodigiosus</i> ; 10 bacteria became
	<i>a</i> (8 days.)	<i>b</i> (5 days.)	
Not treated.....	1,280	67	1,700
Not treated	1,300	58	—
Treated with carbon bisulphide	1,340	—	—
Treated with chloroform.....	1,690	1 020	9,880
Treated with chloroform vapour.....	1,540	920	—
Treated with toluene.....	—	120	3,160
Treated with ether.....	—	300	4,900
Soil (not pasteurised).....	—	40	—

The general result of these experiments with pasteurised soil, is to show that, in the absence of any possible action of protozoa, etc., the solvent has a decided and considerable action of its own in enabling the bacteria to grow. As the solvent has no action upon the toxin, this can only be brought about by the nutrients being made more available, and is a very strong argument in favour of the idea that the segregation of the *agricere* is the chief action of the solvents or volatile disinfectants.

The Distribution of the Agricere in the Soil, after Treatment with Solvents.—It has already been shown (Experiments iii. and iv.) that soils which have been treated with an anti-septic, yield extracts which are more toxic than those obtained from untreated soils, and that soils give up their heat-toxins more freely after treatment (Experiments xi., xii., xiii.). So far as the extracts are concerned, the quantity of extracting material which has generally been used, viz., 200 c.c. for 200 gr., has ensured a greater diffusion of the toxins than of the nutrients from the soil-particles. In the experiments with heated soils, doubtless the quantity of toxin produced has been so great as to overwhelm the action of the nutrients. Still the fact, that a greater quantity of toxin does diffuse out, shows that the fat-solvent has done something more than

kill off phagocytic protozoa. If the treatment with antiseptics facilitates the diffusion of toxins, surely it will also assist the more rapid diffusion of the nutritive matter.

When the disinfectant is added to the soil in quantity sufficient to soak it thoroughly, it is noticed that, as the fat-solvent evaporates, the *agricere* is partly deposited as a ring upon the containing vessel at the surface of the soil. It is not expected that all the *agricere* is in the extreme upper layers, any more than that one extraction with solvent would be enough to remove all the fatty matter. As a matter of fact, the soil has to be percolated for some time with solvent in order to remove all the *agricere*. Upon moistening the soil with chloroform or ether, and allowing the disinfectant to evaporate, the *agricere* should be chiefly in the upper layers, and the lower strata should be comparatively free from it. Experiment showed that such is the case.

A hundred grams of air-dried soil (No. 1) were placed in a beaker, and soaked with ether (Merck). When the odour had passed off, the layers were carefully abstracted, and weighed (20gr.) portions were moistened with 2 c.c. of a suspension of *Bac. prodigiosus*, and incubated overnight. Next day, the soils were shaken up with water, and dilutions prepared for the bacterial count.

EXPERIMENT XX.

20 gr. of soil at	10 bacteria became
Top	33
Middle.....	100
Bottom.....	165

The comparative poverty of the lower layers of the soil in *agricere*, is shown by the greater increase in the growth of the bacteria.

In another experiment, 500 gr. of a garden-soil were placed in a beaker, and wetted with ether, and the solvent was

allowed to evaporate. When all odour had disappeared, the layers were separated into 100 gr. portions. The five layers were tested in the usual manner, by infecting them with a suspension of *Bac. prodigiosus*, and incubating the portions for 20 hours at 28°. Another set was tested by moistening 20 gr. of the soil with 5 c.c. of sterile water, and incubating at 28° for three days. The numbers of the bacteria in the two sets closely follow one another, and, with the exception of the top soil, which is exceptional*, the results agree with the previous experiment.

EXPERIMENT XXI.

	10 cells of <i>Bac. prodigiosus</i> in 20 hours became	Bacteria in 0.0001 gr. of soil in 3 days became
Top layer	800	225
Second layer.....	375	155
Middle „	525	200
Fourth „	820	350
Bottom „	890	360

Other experiments, made by simply moistening the soils, confirmed these results. Portions of soil, weighing 100 gr., were placed in beakers, and wetted with chloroform, ether, or carbon bisulphide. When all odour of the solvent had disappeared, 20 gr. portions were removed from the top, middle, and bottom. These were exposed to the air to ensure the volatilisation of the last traces of solvent, and were then moistened with 5 c.c. of water, and incubated at 28° in a moist atmosphere.

* This exceptional behaviour was confirmed in another experiment with garden-soil, in which 10 cells became 582, 252, and 450 for the top, middle and lower layers respectively. It has also been found in soils exceedingly rich in agricere, *e.g.*, sewage-sick soils, that the top layers are, after treatment, much more nutritive to *Bac. prodigiosus* than the natural spore-forming soil-bacteria which show most growth in the lower layers. The reason for this difference will probably be found to be that, in the rich soils, the action of the toxins is of more moment than the agricere, so far as the growth of the comparatively strong toxin-sensitive *Bac. prodigiosus* is concerned.

EXPERIMENT xxii.

	Bacteria in 0.0001 gr.			
Soil	Good,	No.1(old).	Garden.	Garden.
Solvent	Carbon bisulphide.	Ether.	Chloroform.	Chloroform.
Incubation.....	5 days.	6 days	6 days.	20 days.
Top layer.....	26	141	3,420	2,100
Middle layer.....	39	209	4,440	2,200
Bottom layer. . .	47	244	4,940	2,400

Extracts prepared from the different layers of soil after antiseptic treatment, should contain more toxin in the lower than in the upper strata. This proved to be the case. In the following, the usual method of extraction was adopted—500 gr. of soil were moistened in a beaker with ether, and exposed to the air until all odour had gone; the top 200 gr. and the bottom 200 gr. were each treated with 200 c.c. of tap-water in a mortar. After an hour, the extracts were filtered through paper, and then through porcelain. The extracts were diluted with an equal volume of sterile tap-water, and 10 c.c. portions of full and half strength extract were seeded with 1 c.c. of a suspension of *Bac. prodigiosus*, and incubated overnight.

EXPERIMENT xxiii.

	1,000 bacteria became		Steamed for an hour on four consecutive days.	
Extract of.....	Full strength.	Half strength.	Full strength.	Half strength.
Top 200 gr.	34	190	35	300,000
Bottom 200 gr.	3	80	0	70
Control (tap water)..	137,400		4,300	

The last few experiments show that treatment with fat-solvents brings about an alteration in the soil, whereby the upper layers are less nutritive than the lower, and give up a smaller amount of toxin to an excess of water. The nutritive substances and toxins of the upper layers are less accessible to bacteria, and less easy of extraction than the lower. This is a strong indication that the *agricere* has been translated upwards by the fat-solvent, and, although deposited in a manner quite different from its original condition, is still able to exhibit, though to a less degree, its power of protecting the soil-particles from attack.

Summary.

Rain washes the soil-toxins into the subsoil.

The volatile disinfectants or fat-solvents have no action upon the soil-toxins.

Traces of volatile disinfectants have no action upon the bacteria under experimental conditions.

Substances capable of acting as antitoxins are at present unknown.

The action of heat upon soil is, first, to destroy the original toxin, and then to produce heat toxins, the one action running into the other.

After treatment with volatile disinfectants, the toxins or heated soils are more easily dissolved by water than the toxins of untreated soils.

Treatment with volatile disinfectants induces an increased growth of bacteria in soils in which the protozoa, etc., have been destroyed by moist or dry heat at 65°-75°.

The upper layers of soils which have been treated by volatile disinfectants are less nutritive to bacteria than the lower. Conversely, more toxin is given up to water.

These results point to one of the chief actions of the volatile disinfectants being to translate the *agricere* of the soil, and enable the nutrients to be made more available; that is, they act as fat-solvents.